OCCURRENCE OF STERYL GLYCOSIDES IN EUCALYPT WOOD, KRAFT PULP AND PROCESS LIQUIDS

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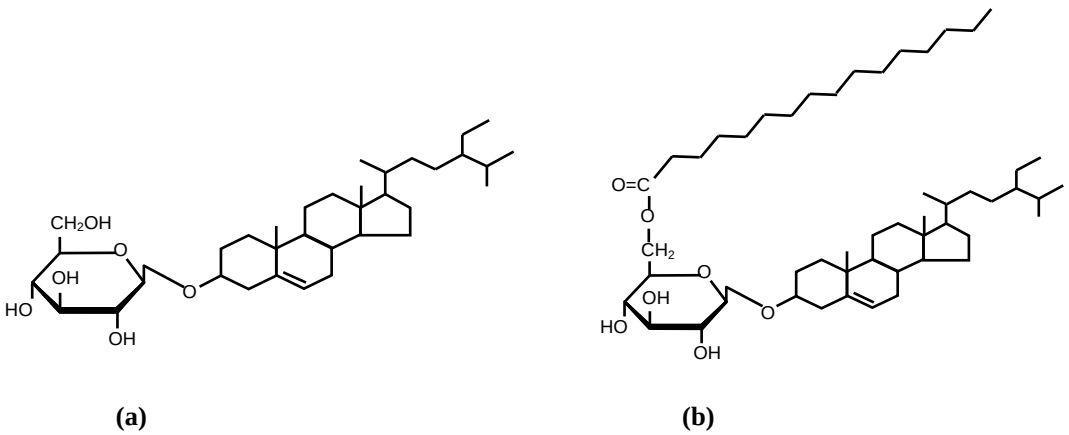
ABSTRACT. The occurrence of steryl glycosides (SG) and acyl steryl glycosides (ASG) in *Eucalyptus globulus* wood was investigated. These compounds were analyzed as their trimethylsilyl ethers by gas chromatography-mass spectrometry (GC/MS). Significant amounts of SG were identified whilst only traces of ASG could be detected. Sitosteryl 3β-D-glucopyranoside and sitosteryl (6´-O-palmitoyl)-3β-D-glucopyranoside were the major SG and ASG found in *E. globulus* wood. The presence of SG and ASG was also investigated after kraft cooking by analyzing unbleached pulp and process water samples. The GC/MS results also revealed the presence of sitosteryl 3β-D-glucopyranoside in these samples. By contrast, no ASG could be detected after kraft cooking.

I. INTRODUCTION

Previous research on *Eucalyptus globulus* wood has shown that free sterols (FS) and esterified sterols (ES) form a considerable percentage of the total lipophilic extractives (1). The composition and fate of sterol lipids from eucalypt wood has been investigated in detail for their relevance in some technical and economic troubles during the production of kraft pulp due to the formation of the so-called pitch deposits (2-6). During wood pulping and refining of pulp, lipophilic extractives (resin) are released forming colloidal pitch, which can deposit on the surface of fibers or equipment being responsible for production troubles (7). In the production of bleached kraft pulp, a large part of the resin originally present in wood is removed during the cooking and bleaching. However, some chemical species survive these processes and are found as pulp extractives, suspended in process waters or forming deposits. On the other hand, other sterol derivatives such as steryl glycosides (SG) and acyl steryl glycosides (ASG) have not been previously identified in eucalypt wood despite they may also have a relevant role in the formation of pitch deposits (Fig. 1). SG have already been found in small amounts in other wood species, such as aspen (8), white oak (9), spruce (10), birch (11,12) and pine (13). However, no studies have been carried out so far to investigate the presence of these compounds in eucalypt wood, the most important raw material for paper pulp production in Spain and many other countries.

In this paper, we report for the first time the occurrence of steryl glycosides in *E. globulus* wood as well as in pulp and process waters during manufacturing of eucalypt kraft pulp. The analysis of SG and ASG was accomplished by gas chromatography-mass spectrometry (GC/MS) as their TMSi ethers.



(a) **(b)**

Figure 1. Chemical structures of the main SG and ASG identified in woods: (a) sitosteryl 3β-D-glucopyranoside; (b) sitosteryl (6´-O-palmitoyl)-3β-D-glucopyranoside.

II. EXPERIMENTAL

Samples of eucalypt wood, unbleached pulp and process waters. The samples used in this study were provided by the ENCE pulp mill in Pontevedra (Spain). This mill produces paper pulp from *E. globulus* wood by kraft pulping and totally chlorine-free (TCF) bleaching. The trees were cut at an age of 12-14 years, subsequently debarked and ground to sawdust. The unbleached pulp sample was collected after the kraft cooking and several washing stages, and a sample of this washing water was also collected. The wood sawdust and the dried pulp were Soxhlet extracted with acetone for 6 hours. The process water sample was extracted three times in a separatory funnel with a mixture of hexane:acetone (2:1) at pH 12. After the extractions, the solvents were evaporated to dryness under vacuum. The dried extracts were silylated with bis(trimethylsilyl)trifluoroacetamide (BSTFA) in the presence of pyridine at 80°C for 90 minutes before GC/MS analyses.

GC/MS. The analyses were performed on a gas chromatograph coupled to a quadrupole mass spectrometer detector equipped with a fused silica capillary column (DB-5HT, J&W; 15 m x 0.25 mm i.d., 0.1 μm film thickness). The oven was heated from 120°C (1 min) to 380°C (5 min) at 10°C/min. The injector (split-splitless) and transfer line temperatures were set at 300°C and 350°C respectively. Helium was used as carrier gas and the injection was performed in splitless mode. The compounds were identified by comparing the retention times and mass spectra obtained with those of authentic standards. A mixture of the standard compounds with a concentration range between 0.1 and 1 mg/mL was used to elaborate a calibration curve for their quantitation. All peaks were quantified by area.

III. RESULTS AND DISCUSSION

The analysis of SG and ASG, as their TMSi ethers, was accomplished by GC/MS using medium-length high-temperature capillary columns with thin films (14). This method enables the elution and separation of high molecular mass lipids. The total-ion chromatogram of the lipid extract of *E. globulus* wood after BSTFA derivatization is shown in Fig. 2. Free and esterified sterols are among the major lipophilic compounds in eucalypt wood. In both cases, the main sterol identified was sitosterol, followed by stigmastanol, and minor amounts of fucosterol, citrostadienol, cycloartenol, 24-methylenecycloartanol and campesterol (1).

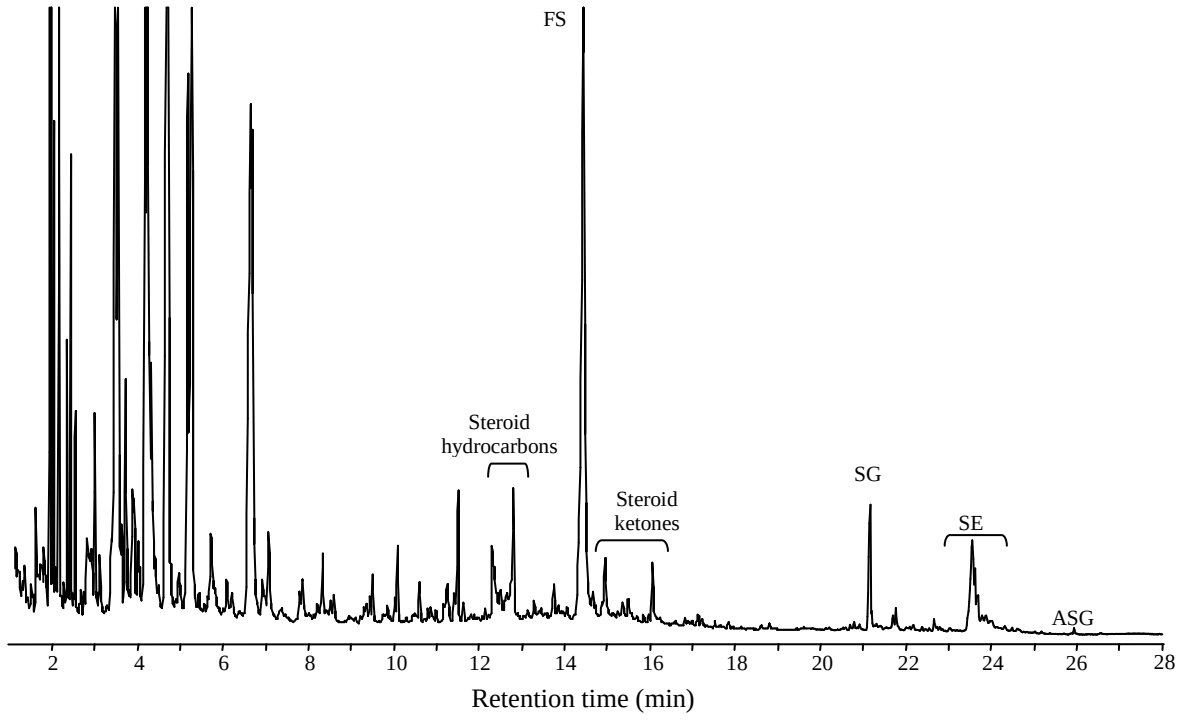


Figure 2. Total-ion chromatogram of the acetone-soluble extract of *E. globulus* wood (after BSTFA derivatization). The inset shows a partial GC/MS trace from a more concentrated sample to show the ASG peaks.

Peaks corresponding to SG and ASG were identified in the high molecular mass region of the chromatogram only after BSTFA derivatization of the *E. globulus* wood extract. The identification of SG and ASG has been accomplished by comparison with the mass spectra and relative retention times of authentic standards. The mass spectrum and relative retention time of the SG peak present in *E. globulus* wood is identical to that of the sitosteryl 3β-D-glucopyranoside standard (Fig. 1a).

It is interesting to note that other sterols present in eucalypt wood in both free and esterified form - such as stigmastanol, citrostadienol, cycloartenol and 24-methylenecycloartanol - have not been found forming SG. On the other hand, the sugar moiety of the SG from *E. globulus* wood consisted of D-glucose, which has been found to be the most important sugar in glycosylated plant sterols (15,16), although several other α- or β-linked glycosyl residues have also been reported (including β-D-galactopyranosyl, β-D-glucuronopyranosyl, α-L-rhamnopyranosyl, α-D-riburonofuranosyl, β-D-xylopyranosyl and α-D-xyluronopyranosyl residues) (15).

ASG were also identified in the acetone extracts of *E. globulus* wood, although in lower amounts (20 mg/Kg wood) than SG (130 mg/Kg wood). The retention time as well as the mass spectrum of the ASG peak present in eucalypt wood was identical to that of sitosteryl (6´-O-palmitoyl)-3β-D-glucopyranoside (Fig. 1b). It is interesting to point out that only palmitic acid has been found esterified to the glucose moiety in the ASG identified in eucalypt wood. The ASG fractions from various tissues of vascular plants usually contain a number of typical saturated and unsaturated fatty acids, mainly C16 and C18. Palmitate, oleate, linoleate, linolenate and stearate are present most frequently. However, it has been reported that in some plants the acyl components of ASG consisted exclusively of saturated fatty acids, mainly palmitic acid (17).

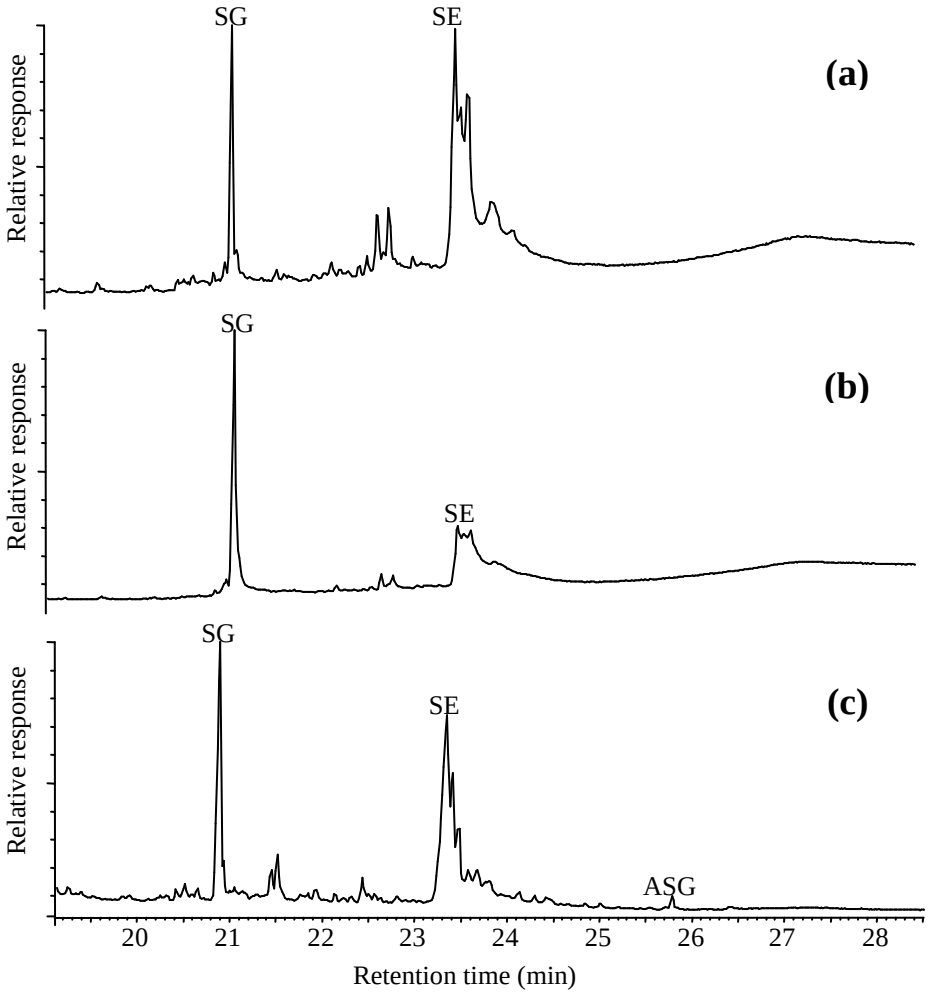


Figure 3. Partial gas chromatograms showing the distribution of SG, SE and ASG in the extracts from process water (a) and unbleached pulp (b) from *E. globulus* kraft cooking compared with *E. globulus* wood extract (c) (after BSTFA derivatization).

According to Nilvebrant and Byström (12) about half of the SG originally present in wood can survive the kraft cooking conditions and be found intact in the pulp. Therefore, in the present work, we have also investigated the occurrence of SG and ASG in process waters and unbleached pulp after eucalypt kraft cooking. Figure 3 shows a region of the total-ion gas chromatograms of the silylated extracts from the process water and unbleached pulp samples selected for this study. Significant amounts of SG (70 mg/10 L in process water and 110 mg/Kg in unbleached pulp) were identified in all samples, with a composition similar to that from eucalypt wood. In all cases, the main peak was identified as sitosteryl 3β-D-glucopyranoside (Fig. 1a). By contrast, no ASG could be detected in pulp and process water samples. The importance of the presence of SG after kraft pulping is due to their high hydrophilic-lipophilic balance, high melting point and very low solubility in water, alkali and the usual organic solvents (7). Due to these properties, SG constitute a part of protecting layers which prevent the cooking and bleaching chemicals to reach the resin and thereby keep them and other extractives in the pulp (12).

IV. ACKNOWLEDGEMENTS

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